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APPLIED ISSUES

The effect of habitat-specific sampling on biological assessment of water quality using a predictive model

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SUMMARY

1. Detection of impairment in macroinvertebrate communities using rapid biological assessment depends on the ability to compare sites, with confidence that differences obtained result from water quality. However, collections from more than one habitat type may introduce variation that can potentially mask water quality differences among sites. Data were collected from the riffle, edge, pool-rock and macrophyte habitats at reference (minimally disturbed) and test (disturbed) stream sites throughout the Australian Capital Territory. The effect of habitat-specific sampling on predictive models for detecting impairment in macroinvertebrate communities was determined. Four models were used: riffle only, edge only, each habitat as an individual object, and all habitats sampled at a site considered as a composite sample.

2. Macroinvertebrates from individual habitats generally clustered into separate groups because collections from the same habitat at different sites were more similar than collections from different habitats within a site. Thus, in the habitats as individual objects model, the taxa predicted to occur at a test site may be an indication of habitat type rather than water quality. The outputs of the composite habitats and riffle and edge models were similar. However, the variable number of habitats included at each site in the composite model may confound the detection of biological impairment because of unequal sampling effort. The riffle and edge models were the most robust because they were less confounded by inter-habitat variation and were based on comparisons made between equivalent environmental units.

3. Comparison of observed/expected taxa ratios for test sites showed that each model could detect biological impairment, indicating considerable data redundancy was introduced by sampling several habitats. In particular, the pool-rock and macrophyte habitats contributed no information with regard to macroinvertebrate taxon occurrence or detection of biological impairment that could not be obtained from either the riffle or edge habitats within the study area.

Introduction

There are several approaches to biological assessment of water quality that use rapid, semi-quantitative collection and processing techniques for stream invertebrates (Resh & McElravy, 1993; Ghetti & Ravera, 1994). Two of the better known techniques are the United States Environmental Protection Agency's (USEPA) Rapid Biological Assessment Protocols (Plafkin *et al.*, 1989) and the British Institute of Freshwater Ecology's RIVPACS (River Invertebrate Prediction and Classification Scheme) predictive model (Wright *et al.*, 1984; Moss *et al.*, 1987; Wright, Furse & Armitage, 1994), both of which have been applied successfully in individual states (U.S.) and on a nationwide scale (U.K.) to assess the impact of human activities on aquatic ecosystems.

Fundamental to the applicability of rapid biological

assessment is the ability to detect impairment of macroinvertebrate communities (undesirable changes brought about by degradation in water or habitat quality), through comparisons with communities from reference sites representing natural or near natural conditions (Resh & Jackson, 1993). However, comparisons are potentially confounded by the many factors which influence the spatial distribution and abundance of aquatic invertebrates (e.g. longitudinal gradients: Vannote *et al.*, 1980; substratum: Minshall, 1984; hydraulic stress: Statzner & Higler, 1986). Rapid biological assessment attempts to account for faunal variation by limiting comparisons to areas with equivalent environmental characteristics. In the USEPA approach, large-scale variation is accounted for by dividing geographical areas into 'ecoregions' or 'sub-ecoregions' thought to have somewhat uniform characteristics (Omernik, 1995), and local variation is accounted for by limiting collection of biota to the riffle zone only (Plafkin *et al.*, 1989). In the British RIVPACS approach (Wright *et al.*, 1984; Moss *et al.*, 1987) reference sites are classified into groups based on homogeneity of their fauna and the physical and chemical characteristics that best describe variation among the groups are determined. These characteristics are then used to predict the macroinvertebrate communities expected to occur at new sites in the absence of environmental stress.

When sites are compared using samples from multiple habitats, confidence is needed that inter-habitat variation is not mistaken for biological impairment. The USEPA approach to biological assessment uses single habitat sampling in an attempt to limit the potential effect of inter-habitat variation on the calculation of biotic indices used to determine impairment (Plafkin *et al.*, 1989). On the other hand, the RIVPACS approach collects from all the major habitats at a site (riffle, margins, pools and macrophytes) in proportion to their occurrence (Furse *et al.*, 1981) and the macroinvertebrates are composited to a single sample which is then searched to obtain a comprehensive species list for the site (Wright *et al.*, 1984). The choice of this method was fuelled by an early objective of the program which was to classify sites according to their macroinvertebrate fauna, for conservation purposes (Wright *et al.*, 1994). In using multi-habitat sampling for water quality assessment, the taxa collected from a site may be weighted to the spatially dominant habitat and sites may tend to be classified because of the particular habitat type represented, rather than water quality or general site features. This is important because it has been shown that macroinvertebrate communities collected from the same habitat at different sites can be more similar than those collected from different habitats at an individual site (Jenkins, Wade & Pugh, 1984; McCulloch, 1986; Brown & Brussock, 1991). Thus, in making comparisons among sites in rapid bioassessment, composite collections from different habitats may introduce an element of inter-habitat variation that can potentially mask water quality differences between sites. Alternatively, sampling habitats separately may produce redundant data that could otherwise have been obtained from sampling one habitat (such as the commonly used riffle), at a reduced cost and effort.

In Australia, a nationwide study known as the National River Health Program (NRHP) has recently been implemented, and aims to develop RIVPACS-style predictive models suitable for use in Australian routine monitoring programmes. The NRHP stipulates the use of habitat-specific sampling, whereby the major habitats at a site (riffle, edge, pool rocks, logs and macrophytes) are sampled separately for macroinvertebrates (e.g. Chessman, 1995). However, the implications of habitat-specific sampling for water quality assessment using predictive models have not been assessed. The present study aimed to investigate the effects of habitat-specific sampling on building and applying the RIVPACS-style model for detecting biological impairment, and to identify redundant data that may be produced by habitat-specific sampling.

Materials and methods

Site selection

Sixty sites, comprising fifty reference (i.e. minimally disturbed) and ten test (i.e. disturbed) sites, were chosen in the Australian Capital Territory (ACT) and surrounding region. The determination of sites as reference or test was based on a recent rapid biological assessment study conducted by Norris (1994) at 100 sites in the region. Of these 100 sites, thirty were classified as reference sites based on their faunal and environmental characteristics. A further twelve reference sites were chosen on the basis of evidence suggesting minimal disturbance, such as a forested

Table 1 Description of test sites used in the predictive model. For habitats sampled, R = riffle, E = edge, P = pool rocks and M = macrophytes. DFS = distance from source. Lake Tuggeranong, Lake Ginninderra and Lake Burley Griffin are artificial reservoirs partly designed as settling ponds for urban runoff, before release into the Murrumbidgee River, which is the major river draining the whole of the study area

Site	Location	Habitats sampled	Stream order	DFS (km)	Dominant surrounding landuse	Disturbance(s)
040	Murrumbidgee River at Angle Crossing	R,E,M	6	180	Cattle/sheep grazing	Diffuse agricultural runoff
047	Murrumbidgee River at Kambah Pool	R,E	6	209	Recreation, residential	Urban runoff via Tuggeranong Creek inflow 4 km upstream; diffuse agricultural runoff
049	Murrumbidgee River at Uriarra Crossing	R,E,P	6	233	Sheep/cattle grazing, pine forestry	Diffuse agricultural and pine forestry runoff
053	Murrumbidgee River at Halls Crossing	R	7	254	Sheep/cattle grazing	Sewage treatment plant 15 km upstream; diffuse agricultural runoff
058	Tuggeranong Creek downstream of dam	R,E	3	14	Residential	Urban runoff via Lake Tuggeranong, site located 500 m downstream of dam wall
061	Tuggeranong Creek upstream of urban area	R,E	2	4	Sheep/cattle grazing	Diffuse agricultural runoff
064	Ginninderra Creek at Latham	R,E	4	20	Residential	Urban runoff via Lake Ginninderra 4 km upstream
070	Molonglo River at Coppins Crossing	R,E	5	93	Sheep/cattle grazing, pine forestry	Urban runoff via Lake Burley Griffin 8 km upstream; hypolimnetic release from Lake Burley Griffin
078	Molonglo River at Bungendore Road	R,E,M	6	35	Sheep/cattle grazing	Trace metal pollution from an abandoned mine site 27 km upstream
109	Queanbeyan River at Wickerslack	R,E,P	5	77	Light residential, recreation	Hypolimnetic release from Googong Reservoir 3 km upstream

catchment, remoteness or previous faunal sampling. The remaining eight reference sites were chosen to represent lowland rivers in agricultural areas. Some difficulty was encountered in objectively choosing minimally impaired reference sites for agricultural areas because of the potential for disturbance to have occurred previously, and sites were chosen on the basis of invertebrate taxa collected from them by Norris (1994), or on conditions such as upstream land use, extensive riparian vegetation and the absence of obvious point source impacts.

Test sites (Table 1) were selected to include the range of environmental characteristics found at the reference sites, based on the classification groups obtained by Norris (1994). Consideration was given to previous studies detailing disturbances (Norris, 1986; Hogg & Norris, 1991) and sites were also selected to represent a range of disturbance types known or suspected to be occurring in rivers of the ACT region (Table 1).

Invertebrate sampling

At each site, macroinvertebrates were sampled separately from four habitats: riffle (R), edge/backwater (E), macrophytes (M) and pool rocks (P), where these habitats occurred within a 100 m reach of the river. Riffle macroinvertebrates were collected by kick sampling using a triangular frame sweep net (250 μm mesh, aperture 300 mm at bottom edge). Edge macroinvertebrates were collected from within 1 m of the bank by vigorously sweeping the same net along river margins, in areas of little or no flow. Areas devoid of bank vegetation or overhangs were avoided. Macroinvertebrates were collected from macrophytes using the same sweeping technique employed in the marginal areas. A 10 m sampling transect was used for each of these habitats; where insufficient area was available the length of transect sampled was recorded. Collection of macroinvertebrates from pools was lim-

ited to wadeable areas containing rocks which could be dislodged easily and carried to the bank, hence this habitat is termed pool rocks. A range of boulder-, cobble- and pebble-sized rocks was removed from an area with little or no flow. Rocks were placed in a plastic tray and 100 invertebrates removed using forceps, with care being taken to select as many taxa as possible. Macroinvertebrates were preserved in the field using 10% formalin, with Rose Bengal stain added to aid sorting. All sampling was conducted in April 1994.

In the laboratory, each preserved sample (excluding pool rocks) was placed in a subsampling box with 100 cells (Marchant, 1989) and mixed until evenly distributed. Cells were selected using a random number table and their contents transferred into a flask using a vacuum pump. Initially, a modest number of cells (1–10) was selected, with the aim of obtaining 200 individuals, and all invertebrates were removed from the selected cells. This standardization of subsampling enables calculation of total macroinvertebrate abundances, proportional to their occurrence in the subsample collected. Invertebrates were identified to family level using the keys listed by Hawking (1994). Exceptions to this were Chironomidae, which were identified to subfamily, and worms (Oligochaeta), mites (Arachnida), turbellarians (Turbellaria) and leeches (Hirudinea), which were identified to class. Identifications were verified against a reference collection of specimens held at the Cooperative Research Centre for Freshwater Ecology, University of Canberra.

Physical, chemical and habitat sampling

The environmental variables measured at each site are listed in Table 2. The habitat assessment variables (Table 2) were derived from those originally used in the U.S. Rapid Bioassessment Protocols (Plafkin *et al.*, 1989). Substratum characteristics (Table 2) were assessed visually along the 10 m transect used for macroinvertebrate sampling, in each habitat type.

Analysis

The effect of habitat-specific sampling on detection of biological impairment was examined using three analytical treatments, which were used to construct four different models of the type described by Wright *et al.* (1984) and Moss *et al.* (1987).

1 Habitat type considered separately. This treatment was used to construct models for the riffle (forty-six reference sites) and edge (forty-two reference sites) habitats. The other habitat types were not represented at sufficient sites to build individual models (twenty pool-rock reference sites, eleven macrophyte reference sites).

2 Each habitat sampled within a site considered as an individual object, within a single analysis. This treatment contained 119 reference objects that were used to construct the model, each of which corresponded to one habitat sampled within a site.

3 All habitats sampled at a site considered as a composite sample. This treatment contained fifty reference sites and because of the uneven number of habitats sampled at a site, the taxa counts for each habitat at a site were combined and converted to proportions of the total. The model from this treatment used 106 environmental variables including those measured for the whole site, plus each measurement taken from each habitat type within a site (Table 2). This treatment broadly corresponds to the method used by Wright *et al.* (1984) in developing the British RIVPACS model, except that in the present study habitats were not sampled in proportion to their spatial occurrence at a site, and proportional abundance rather than presence/absence data were used.

Several studies have shown that most of the patterns of distribution revealed in multivariate analyses of benthic macroinvertebrate communities are the result of the contribution made by the common taxa (e.g. Norris, Lake & Swain, 1982; Marchant, 1990). Thus, when all habitats within a site were considered together, taxa that occurred at five or less (10%) of the reference sites were removed. This resulted in a set of fifty-six commonly occurring taxa (Table 3).

Reference sites were classified into groups based on their faunal composition using the flexible unweighted pair-groups and arithmetic averages (UPGMA) fusion strategy recommended by Belbin & McDonald (1993). The Bray and Curtis association measure was used, following the recommendation of Faith, Minchin & Belbin (1987). Faunal data were not transformed because the subsampling procedure provided proportional representation (numbers per 200 animals) and it was desired that weight be given to numerically dominant taxa. Groups were selected by viewing a dendrogram representation of the classification.

Macroinvertebrate community structure at reference

Table 2 Environmental variables measured at reference and test sites in the study region

Riparian zone composition	
treesgt	% cover of trees greater than 10 m in height
treeslt	% cover of trees less than 10 m in height
shrubs	% cover of shrubs
grasses	% cover of grasses
ripwidth	Width of riparian zone (m)
Substratum characteristics	
bedrock*	% bedrock
boulder*	% boulder (>256 mm)
cobble*	% cobble (64–256 mm)
gravel*	% gravel (2–64 mm)
sand*	% sand (0.06–2 mm)
silt*	% silt (0.004–0.06 mm)
clay*	% clay (<0.004 mm)
macro*†	% macrophytes
detrit*	% cover of substrate by detritus
muckmud*	% cover of substrate by muck and mud
peri*	% cover of substrate by periphyton
slimes*	% cover of substrate by slimes
filamen*	% cover of substrate by filamentous algae
Habitat assessment	
scoursc§	Scouring and deposition score
vegsc§	Bank vegetative stability score
sidesc§	Dominant streamside vegetation score
channsc§	Channelization score
velocsc§	Velocity/depth category score
priffsc§	Habitat variety score
banksc§	Bank stability score
bottomsc§	Bottom substrate score
embedsc§	Embeddedness score
habscore§	Total habitat score
Physical and chemical characteristics	
storder	Stream order
alt	Altitude (m)
dfs	Distance from source (km)
lat	Latitude
long	Longitude
watwidth	Stream width (water)
bnkwidth	Stream width (channel)
bheight	Bank height (m)
slope	Slope (cm m^{-1})
temp	Temperature (°C)
cond	Conductivity (μS cm^{-1})
ph	pH
do	Dissolved oxygen (mg l^{-1})
dosat	O_2% saturation
tn	Total nitrogen (mg l^{-1})
nox	Nitrates/nitrites (mg l^{-1})
tp	Total phosphorus (mg l^{-1})
alk	Alkalinity (mg l^{-1} $CaCO_3$)
turb	Turbidity (FNU)
depth*	Mean depth (cm)
veloc*	Velocity (m s^{-1})
pcriff	% riffle in 100 m transect
pcedge	% edge in 100 m transect
pcpool	% pool in 100 m transect
pcmacro	% macrophytes in 100 m transect
Pool rocks only	
ptotrock‡	No. of rocks used for live pick

*Variables measured at each habitat. Where these are used within the same analysis, variables are preceded by an R,E,P or M to distinguish habitat type.
†This was included to distinguish macrophytes as a discrete substrate type and was always scored as 0% for the riffle, edge and pool-rock habitats and as 100% for macrophytes.
‡Used in composite habitats analytical treatment only.
§Habitat assessment scores following Plafkin *et al.* (1989).

sites was ordinated using hybrid multi-dimensional scaling (HMDS; Belbin, 1992). A Monte Carlo simulation (MCSS with 100 permutations; Belbin, 1992) was performed on the invertebrate ordinations to determine the probability of the ordination having explained structure in the data that might have occurred by chance alone (Faith, 1990). The relationship between environmental variables and the position of sites in invertebrate ordination space was determined using principal axis correlation (PCC; Belbin, 1992; see also Faith & Norris, 1989). Monte Carlo significance tests (MCAO with 100 permutations; Belbin, 1992) were performed to test the significance of the correlation values obtained in the PCC procedure, so that a subset of variables most closely associated with the structure of the invertebrate data could be selected. Only those environmental variables with a significance of 0.05 or better were considered important.

The selection of environmental variables important in structuring the invertebrate data was corroborated using a stepwise discriminant function analysis (DFA) (PROC STEPDISC; SAS Institute, 1988). By entering variables from the data set one at a time, this analysis selects the physical, chemical or habitat variables which are best able to discriminate among the groups of sites formed by classification of invertebrates. The significance level for variables to enter and to stay in the stepwise DFA were both set at 0.05.

Table 3 Summary of macroinvertebrate taxon occurrence across the four habitats sampled. Symbols indicate that the taxon occurred at one or more reference (●) or test (○) sites

Taxa	Used in analysis	Riffle		Edge		Pool rocks		Macrophytes	
		Ref. (n = 46)	Test (n = 10)	Ref. (n = 42)	Test (n = 9)	Ref. (n = 20)	Test (n = 3)	Ref. (n = 11)	Test (n = 2)
Annelida									
Oligochaeta	Y	●	○	●	○	●		●	
Hirudinea				●	○				
Platyhelminthes									
Turbellaria	Y		○			●	○	●	
Hydracarina									
Hydracarina	Y	●		●		●			
Bivalvia									
Sphaeriidae	Y	●		●	○				
Corbiculidae	Y	●	○	●				●	
Gastropoda									
Planorbidae	Y	●		●	○	●		●	○
Ancylidae	Y	●		●		●	○		
Lymnaeidae	Y	●		●	○			●	
Succineidae				●					
Hydrobiidae					○				
Crustacea									
Amphipoda	Y	●	○	●	○	●		●	
Atyidae	Y		○	●	○			●	○
Palaemonidae				●	○				
Copepoda	Y	●		●	○			●	
Coleoptera									
Elmidae (adult)	Y	●		●		●			
Elmidae (larvae)	Y	●	○	●		●	○	●	
Hydrophilidae	Y	●	○	●	○	●		●	
Psephenidae	Y	●	○	●		●	○	●	○
Scirtidae	Y	●	○	●	○			●	
Curculionidae		●							
Noteridae (adult)				●					
Gyrinidae (larvae)				●		●			
Gyrinidae (adult)				●					
Staphylinidae				●					
Collembola									
Collembola	Y			●	○	●		●	
Lepidoptera									
Pyralidae	Y	●	○	●		●		●	○
Diptera									
Athericidae	Y	●		●	○			●	
Empididae	Y	●	○	●		●			
Simuliidae	Y	●	○	●	○	●		●	○
Tipulidae	Y	●	○	●	○	●		●	
Ceratopogonidae	Y	●		●	○	●		●	
Tabanidae		●							
Psychodidae		●							
Culicidae				●	○			●	
Dixidae	Y			●	○				
Chironominae	Y	●	○	●	○	●	○	●	
Tanypodinae	Y	●	○	●	○	●		●	
Orthocladiinae	Y	●	○	●	○	●	○	●	○
Podonominae	Y	●	○	●		●		●	
Diamesinae		●	○	●					
Chironomidae (pupae)	Y	●	○	●	○	●		●	○

Table 3 *Continued*

Taxa	Used in analysis	Riffle		Edge		Pool rocks		Macrophytes	
		Ref. (n = 46)	Test (n = 10)	Ref. (n = 42)	Test (n = 9)	Ref. (n = 20)	Test (n = 3)	Ref. (n = 11)	Test (n = 2)
Ephemeroptera									
Baetidae	Y	●	○	●	○	●	○	●	○
Caenidae	Y	●	○	●	○	●	○	●	
Coloburiscidae	Y	●		●		●			
Leptophlebiidae	Y	●	○	●	○	●	○	●	
Megaloptera									
Corydalidae	Y	●	○	●				●	
Neuroptera									
Osmylidae				●					
Odonata									
Gomphidae	Y	●	○	●	○	●		●	
Aeshnidae	Y	●		●	○			●	○
Corduliidae	Y			●		●		●	
Synlestidae	Y			●	○	●		●	
Lestidae	Y			●				●	
Coenagrionidae	Y			●	○			●	○
Amphipterygidae						●			
Megapodagrionidae						●			
Plecoptera									
Austroperlidae	Y	●		●		●		●	
Eustheniidae		●							
Gripopterygidae	Y	●	○	●	○	●		●	
Notonemouridae				●					
Hemiptera									
Corixidae (adult)	Y	●	○	●	○	●		●	○
Notonectidae (adult)	Y			●	○			●	○
Gerridae (adult)				●					
Mesoveliidae (adult)				●					
Trichoptera									
Conoesucidae	Y	●	○	●		●		●	
Calamoceratidae	Y	●		●	○	●		●	
Ecnomidae	Y	●	○	●	○	●	○	●	
Glossosomatidae	Y	●		●		●			
Helicopsychidae	Y	●		●		●		●	
Hydrobiosidae	Y	●		●		●		●	
Hydropsychidae	Y	●	○	●	○	●	○	●	
Hydroptilidae	Y	●	○	●	○	●	○	●	○
Leptoceridae	Y	●		●	○	●		●	○
Philopotamidae	Y	●		●		●			
Odontoceridae		●		●		●			
Polycentropodidae	Y	●		●		●			
Tasimiidae	Y	●		●		●			
Philorheithridae				●				●	
Atriplectidae				●				●	
Limnephilidae								●	
Helicophidae				●					
No. of taxa		53	30	72	38	47	12	49	14

The set of environmental variables found to be significant in the PCC procedure and the set of environmental variables from the stepwise DFA were then used in a DFA (PROC DISCRIM CROSSVALIDATE; SAS Institute, 1988) to predict into which invertebrate site group a reference site would fall based on the limited set of environmental variables. After testing several combinations of environmental variables from

the PCC and stepwise DFA analyses, the combination which produced the lowest error in predicting group membership of a site in the DFA was selected as the set of variables to be used in the predictive model.

Environmental and macroinvertebrate data collected in the present study were used to construct predictive models following the methods described by Wright *et al.* (1984) and Moss *et al.* (1987). The deviation between the number of taxa expected (calculated by the model) and the number of taxa that were observed (collected in the field) is expressed as the ratio of observed/expected taxa, and used as an indication of biological impairment at a test site (Wright *et al.*, 1994). This ratio, expected to be close to 1 in the absence of biological impairment, was used as the measure by which the effects of habitat-specific sampling were determined in this study. The allocation of observed/expected ratios to biological bands follows the categories used by Wright *et al.* (1994). Band A (observed/expected ratio > 0.79) is equivalent to reference conditions and bands B (0.58–0.78), C (0.37–0.57) and D (< 0.37) correspond to progressively more impaired conditions. The locations of band boundaries have received little testing in Australia and therefore are not definitive. They are used in this study for ease of interpretation in relation to biological impairment at a test site.

Observed/expected taxa ratios of the test sites were compared, to test for differences among the model predictions. Comparisons were done using a Wilcoxon signed ranks test with a Sidak correction for multiple comparisons (Sokal & Rohlf, 1981). Observed/expected ratios from each model were tested against all others, giving a total of eight comparisons. These were:

1 riffle-only model *v* edge-only model;
2 riffle-only model *v* riffle sites from the habitats as individual objects model;
3 edge-only model *v* edge sites from the habitats as individual objects model;
4 riffle sites from the habitats as individual objects model *v* edge sites from the same model;
5 riffle-only model *v* sites from the composite habitats model;
6 edge-only model *v* sites from the composite habitats model;
7 riffle sites from the habitats as individual objects model *v* sites from the composite habitats model;
8 edge sites from the habitats as individual objects model *v* sites from the composite habitats model.

Wright *et al.* (1984) and Moss *et al.* (1987) arbitrarily chose two levels at which to determine observed/expected taxa ratios: taxa with a probability of occurrence within reference site groups of greater than 50% and greater than 75%. In this study, observed/expected taxa ratios were determined at the level of 50%, which has been shown to be an accurate measure of water quality (Wright, 1995). The accuracy of the predictive models developed in the present study in determining biological impairment was validated for the riffle and edge habitats separately, by randomly removing five reference sites from the data set. The remaining reference sites were reanalysed, using the same technique described above, and the five selected sites were then substituted into the new model as test sites.

Results

Distribution of macroinvertebrates among habitats

More taxa were collected from the edge habitat than from the riffle, pool-rock or macrophyte habitats at both the reference and test sites (Table 3). As would be expected, the number of taxa collected at test sites was low, relative to reference sites (Table 3). Most taxa collected from the edge habitat were also found in the riffle, exceptions being the coleopteran families Noteridae, Gyrinidae and Staphylinidae (semi-aquatic), several damselfly families, the dipteran family Dixidae and the hemipteran families Notonectidae, Gerridae and Mesoveliidae (Table 3), all of which prefer slowly flowing waters (Williams, 1980). Pool-rock fauna was similar to that collected from the riffle but included the gyrinids and the corduliid and synlestid Odonata which were otherwise only collected from the edge habitat (Table 3). The fauna collected from macrophytes was generally similar to that collected from the edge habitat (Table 3). Among the reference sites, sixteen taxa were exclusive to one habitat: ten to the edge, three to the riffle, two to the pool rocks and one to the macrophytes (Table 3).

Predictive model

Selection of habitat variables. For each of the analytical treatments, the error in allocating sites to the predefined groups in the DFA was lower for the set of environmental variables selected from the stepwise DFA than for those selected from the PCC procedure

Table 4 Comparison of discriminant function analyses (DFA) for the riffle, edge, each habitat as an individual object and composite habitats analytical treatments, using sets of environmental variables significant ($P < 0.05$) in the principle axis correlation (environmental variables in invertebrate space) and in the stepwise DFA. DFA and model refers to the set of variables which were used in the predictive model. Error refers to the percentage of sites which were misclassified into groups in the DFA using the cross-validation option. An explanation of variable codes is given in Table 2

	PCC	Stepwise DFA	DFA and model
Riffle-only treatment*	storder, alt, dfs, watwidth, bnkwidth, bheight, grasses, ripwidth, temp, cond, ph, do, alk, peri, velocsc, priffsc, sidesc, habscore	silt, ripwidth, do, watwidth, alt, bottomsc, pcmacro	silt, ripwidth, do, watwidth, alt, bottomsc, pcmacro, storder, dfs, alk, habscore
	Error = 53%	Error = 51%	Error = 24%
Edge-only treatment*	storder, alt, dfs, long, watwidth, bnkwidth, slope, ripwidth, alk, pcriff, pcpool, veloc, sidesc	peri, detrit, long, boulder, muckmud	peri, detrit, long, boulder, muckmud, sidesc, veloc
	Error = 58%	Error = 38%	Error = 32%
Habitats as individual objects treatment*	storder, alt, dfs, long, watwidth, bnkwidth, bheight, shrubs, temp, ph, dosat, pcmacro, cobble, banksc	veloc, alt, long, detrit, gravel, cobble, alk, embedsc	veloc, alt, long, detrit, gravel, cobble, alk, embedsc
	Error = 57%	Error = 26%	Error = 26%
Composite habitats treatment*	storder, alt, dfs, long, watwidth, bnkwidth, bheight, ripwidth, temp, cond, ph, alk, pcriff, pcpool, pcmacro, rsilt, rperi, rdepth, rveloc, eboulder, egravel, edetrit, eperi, ptotrock, mdetrit, mmuckmud, mdepth, mveloc, velocsc, priffsc, banksc, sidesc	mveloc, watwidth, cond, alt, pcriff, eveloc, alk, bheight, eslimes, do, rsand, bottomsc, rgravel, rbedrock, pdetrit, dosat	mveloc, watwidth, cond, alt, pcriff, eveloc, alk, bheight, eslimes, do, rsand, bottomsc, rgravel, rbedrock, pdetrit, ptotrock, rveloc, pcpool, temp
	Error = 58%	Error = 32%	Error = 23%

*Monte Carlo testing of hybrid multidimensional scaling ordination scores revealed that stress levels of 0.19 (riffle only), 0.20 (edge only), 0.21 (individual objects) and 0.22 (composite habitats) in three dimensions were all highly significant ($P < 0.01$).

(Table 4). Generally, the errors in predicting group membership of a site using the environmental variables significant in the stepwise DFA could be reduced by selecting additional variables from the PCC list (Table 4). The combination of variables which produced the lowest error in the DFA was used in the predictive model (Table 4; DFA and model column).

Environmental variables selected for use in the models generally included several that were indicators, or surrogates for geographical locationm, such as longitude, stream order, distance from the river source, water width, altitude, alkalinity and water temperature (Table 4). However, of the seven variables selected for use in the edge model, only longitude was an indicator of location (Table 4). Most other variables selected for use in the edge model were those associated with slowly flowing depositional areas and included detritus cover, muck and mud cover, side score from the habitat assessment and velocity (Table 4).

Model validation and comparisons of predictions. The observed/expected taxa ratio for the five reference sites excluded and used for validation ranged from 0.90 to 1.13 for the five riffle sites and from 0.76 to 1.08 for the five edge sites, at the 50% probability

Table 5 Site groups formed by classification of macroinvertebrates collected from riffle (R), edge (E), pool-rock (P) and macrophyte (M) habitats, using three analytical treatments. *Groups that were excluded from further analyses. The total number of reference sites sampled was forty-six at the riffle habitat, forty-two at the edge habitat, twenty at the pool-rock habitat and eleven at the macrophyte habitat

	Number of sites per group					
	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
1 Habitat type considered separately						
Riffle	11	1*	16	18		
Edge	22	7	11	2*		
2 Each habitat considered as an individual object in a single analysis						
	23R	2R	4R	1R*	2E*	16R
		29E	4E	1M*		7E
		6M	14P			4M
						6P
3 All habitats sampled at a site considered as a composite sample						
	4	21	16	2*	7	

level. In all but one case at the edge habitat, the five randomly selected reference sites were equivalent to reference conditions (observed/expected > 0.79) according to the biological bands stipulated by Wright *et al.* (1994). In that single case, however, the observed/expected taxa ratio of 0.76 was close to reference conditions.

There was only one significant difference among the eight comparisons of model outputs. Observed/expected taxa ratios for the edge sites from the habitats as individual objects model were significantly higher than those from the edge-only model ($t_s = 0.0039$, $P < 0.0064$, Sidak correction for eight comparisons).

Riffle and edge habitats considered separately. Based on similarities in the abundance of taxa, classification of the fauna collected from the riffle habitat revealed three distinct site groups (groups 1, 3 and 4; Table 5). One site on a second-order tributary had only 2 m of poor quality riffle available for sampling and a total abundance of forty-seven individuals. This site was placed on its own (group 2; Table 5) and was not included in further analyses because as a single site group it would contain insufficient information to allow accurate prediction of group membership in the DFA. Classification of the fauna collected from the edge habitat revealed three distinct site groups (groups 1, 2 and 3; Table 5). Two sites were placed into a separate group (group 4; Table 5) because of a numerical dominance of amphipods; this small group also was excluded from subsequent analyses.

Based on observed/expected taxa ratios at the 50% probability level, most riffle and edge test sites were placed into biological bands B, C or D (Table 6), which is indicative of impairment at these sites (Wright *et al.*, 1994). The exception to this was riffle test site 061 and edge test sites 040, 061 and 070 which were placed in band A (Table 6), indicating that their faunas were not impaired.

Each habitat considered as an individual object within a single analysis. Each habitat type generally classified into the same group (Table 5), based on similarities in the abundance of taxa collected from reference sites. Consequently, most of the twenty-four riffle, edge, pool-rock and macrophyte test sites were placed into the classification group that had the majority of objects belonging to the corresponding habitat type (Table 7). Eight of the ten riffle test sites had the greatest chance of belonging to group 1 (Table 7), which was comprised entirely of riffle reference sites (Table 5). Similarly, five of the nine edge test sites had the greatest chance of belonging to group 2 (Table 7), which was mainly comprised of edge sites (Table 5). Only one of the pool-rock test sites had the greatest chance of belonging to group 3 which was mostly pool-rock sites (Table 5); the other two were placed into group 2 (Table 7). The two macrophyte test sites had the greatest chance of belonging to group 2 (Table 7), which contained mostly edge habitat reference sites (Table 5). The test sites on Ginninderra Creek (064R and 064E, Table 1) both had

Table 6 Numbers of taxa expected and observed at test sites. Calculations are from the separate riffle habitat and edge habitat models, using taxa with a 50% or better chance of occurring within reference site groups. Observed/expected biological bands coincide with those of Wright *et al.* (1994). A suitable edge habitat was not available at site 053

	Riffle			Edge		
Site	No. taxa observed (O)	No. taxa expected (E)	O/E (band)	No. taxa observed (O)	No. taxa expected (E)	O/E (band)
040	7	9.36	0.75 (B)	12	13.40	0.90 (A)
047	7	9.36	0.75 (B)	10	13.36	0.75 (B)
049	7	9.37	0.75 (B)	9	12.83	0.70 (B)
053	5	9.36	0.53 (C)	–	–	–
058	7	16.11	0.43 (C)	2	14.78	0.14 (D)
061	10	11.78	0.85 (A)	11	13.43	0.82 (A)
064	4	11.70	0.34 (D)	7	14.37	0.49 (C)
070	6	10.41	0.58 (B)	11	13.82	0.80 (A)
078	8	15.87	0.50 (C)	7	12.27	0.57 (C)
109	10	13.39	0.75 (B)	8	13.36	0.60 (B)

Table 7 Numbers of taxa expected and observed at test sites. Calculations are from the habitats as individual objects model, using taxa with a 50% or better chance of occurring within a reference site group. Observed/expected biological bands coincide with those of Wright *et al.* (1994). The reference site group into which each of the test sites was most likely to be placed by the model is given in bold, followed by the percentage probability of the test site belonging to that group

Site	Probability of group membership (%)	No. taxa observed (O)	No. taxa expected (E)	O/E (band)
040R	**1** (99)	7	12.11	0.58 (B)
040E	**2** (100)	12	12.61	0.95 (A)
040M	**2** (90)	4	12.22	0.33 (D)
047R	**1** (91)	9	11.85	0.76 (B)
047E	**2** (100)	10	12.61	0.79 (A)
049R	**1** (99)	7	12.08	0.58 (B)
049E	**2** (96)	10	12.46	0.80 (A)
049P	**3** (98)	4	5.87	0.68 (B)
053R	**1** (100)	5	12.13	0.41 (C)
058R	**1** (42)	8	11.09	0.72 (B)
058E	**3** (94)	2	5.88	0.34 (D)
061R	**1** (93)	10	11.93	0.84 (A)
061E	**2** (70)	9	10.53	0.85 (A)
061P	**2** (89)	5	12.25	0.41 (C)
064R	**6** (100)	4	11.94	0.34 (D)
064E	**6** (100)	6	11.94	0.50 (C)
070R	**2** (49)	8	11.88	0.67 (B)
070E	**2** (99)	11	12.60	0.87 (A)
078R	**1** (100)	8	12.13	0.66 (B)
078E	**3** (58)	8	9.03	0.89 (A)
078M	**2** (88)	7	12.14	0.58 (B)
109R	**1** (100)	9	12.13	0.74 (B)
109E	**3** (69)	6	9.22	0.65 (B)
109P	**2** (66)	6	10.34	0.58 (B)

a 100% chance of belonging to group 6 (Table 7), which contained mostly riffle sites (Table 5).

All but two of the ten riffle and three of the nine edge test sites used in the habitats as individual objects model (Table 7) were placed into the same biological band as in the separate riffle and edge models (Table 6). Three of these sites (049E, 047E and 078R) had only a small change (0.04–0.16) in the observed/expected ratio between the two models (Tables 6 and 7). The other two sites (058R and 078E) had a difference of around 0.3 in observed/expected ratio between the two models (Tables 6 and 7).

The observed/expected ratio and biological band of the two macrophyte test sites and the three pool-rock test sites indicate impairment of fauna at both these habitats (Table 7). Most macrophyte and pool-rock test sites in the habitats as individual objects model were allocated to the same biological band as either the edge or riffle at the same site location (Table 7). Exceptions to this were sites 040M and 061P, both of which had observed/expected ratios much lower than the other habitats at the same location (Table 7).

Habitats within a site considered as a composite sample. Classification of the composite faunal samples revealed four distinct site groups based on similarities in the proportional abundance of taxa (groups 1, 2, 3 and 5; Table 5). Two sites were dominated entirely by macrophytes and formed a small group (group 4; Table 5) which was excluded from subsequent analyses.

Table 8 Numbers of taxa observed and expected at test sites. Calculations are from the composite habitats model, using taxa with a 50% or better chance of occurring within reference site groups. Observed/expected biological bands coincide with those of Wright *et al.* (1994)

Site	No. taxa observed (O)	No. taxa expected (E)	O/E (band)
040	17	18.48	0.92 (A)
047	13	17.50	0.74 (B)
049	15	22.04	0.68 (B)
053	5	17.50	0.29 (D)
058	9	21.30	0.42 (C)
061	17	18.82	0.90 (A)
064	10	22.05	0.45 (C)
070	13	17.50	0.74 (B)
078	15	17.50	0.86 (A)
109	14	22.05	0.63 (B)

Although observed/expected taxa ratios of the ten composite habitats model test sites were not significantly different from any of the other analytical treatments, there was some inconsistency between the ratios obtained from each model. Some test sites were allocated to a higher or lower biological band in the composite habitats model than the corresponding site in the riffle or edge models (Tables 6 and 8). For example, site 053 had only the riffle habitat available for sampling (Table 1) and was allocated to band C in the riffle-only model (Table 6), but to band D in the composite habitats model (Table 8). Similarly, site 078 was allocated to band A in the composite habitats model (Table 8), however, in both the riffle and edge models, this site was allocated to band C (Table 6).

Discussion

The three analytical treatments considered in this study are options for data collection and processing techniques for use in routine monitoring of water quality. However, the suitability of predictive models for monitoring programmes is dependent upon their ease of application and practicality in providing management information at minimal cost and effort. In the present study, each predictive model was effective in detecting biological impairment at test sites, indicating considerable data redundancy when sampling several habitats. Also, the models may be less robust when data from several habitats within a site are combined, having the potential to confound detection of biological impairment.

Habitats as individual objects model

Classification of each habitat as an individual object revealed that groups formed according to habitat type (Table 5) because faunas from the same habitat at different sites were more similar than faunas from different habitats within a site. Several authors have obtained similar findings from classification analysis of faunal assemblages collected at different habitats. Pettigrove (1990) found that pool communities clustered into one group, indicating high faunal similarity among them, and Rutt, Weatherley & Ormerod (1989) also found that riffle and edge faunal communities clustered discretely. Likewise, Jenkins *et al.* (1984) collected macroinvertebrates from riffles, edges, pools and tree roots and found that the majority of riffles clustered together but that the other habitats were dispersed throughout the remaining groups. The implication of habitat types tending to cluster together (Table 5) is that matching of test sites with groups of reference sites will only represent habitat-type differences, rather than general site characteristics. In the present study, test sites were either known or suspected to be disturbed. The habitats as individual objects model placed the majority of test sites into the invertebrate-based classification group that contained sites of the corresponding habitat type (Tables 5 and 7). While this model detected biological impairment of these sites, it has the potential to be confounded because each test site is matched with a group of reference sites using a limited set of environmental variables (Table 4) and may result in a comparison with an inappropriate habitat. Thus, the taxa predicted by the model to occur at a test site may be an indication of habitat type rather than water quality.

Composite habitats model

The relationship between sampling effort and the number of taxa collected has been well documented for benthic macroinvertebrate surveys using dip nets (e.g. Armitage *et al.*, 1983; Mackey, Cooling & Berrie, 1984; Storey, Edward & Gazey, 1991). In the present study, there was some inconsistency between the observed/expected taxa ratios and the biological bands obtained for test sites in the composite habitats model and both the riffle and edge models (Tables 6 and 8). This suggests that detection of impairment may be confounded by the variety of habitats consid-

ered, given that the number of taxa collected will be related to the number of habitats sampled. Thus, the severity of impact may appear more or less pronounced in a model based on composite habitats than when models based on individual habitats such as the riffle or edge are used. In the present study, macroinvertebrates were collected from each habitat separately but an attempt was made to account for the effect of increased sampling effort in the combined samples by using proportional abundance data in the composite habitats model. This is important if the composite habitats model is to be used for routine biological monitoring programmes because if the original data base of reference sites is built on composite samples, new sites can only be tested if they contain biota collected using comparable sampling effort. Therefore, it may be safer to limit collection to one habitat to minimize the effect of unequal sampling effort, such as in the USEPA approach (Plafkin *et al.*, 1989), or to devise a separate predictive model for each habitat.

Riffle and edge models

The USEPA approach to biological monitoring (Plafkin *et al.*, 1989) is based on the principle that variation in the natural distribution of invertebrates is accounted for by limiting comparisons to areas with equivalent environmental characteristics. In the present study, the riffle and edge models produced results that were not confounded by inter-habitat variation (Table 6) because they were based on comparisons made between equivalent environmental units. Both models were equally effective at detecting biological impairment at the test sites. However, the riffle model may be slightly more robust than the edge model because of the greater variability within the edge habitat, as shown by its having more taxa (Table 3), and by the nature of the environmental variables used in the predictive model (Table 4). Most of the predictor variables used in the riffle model were those associated with geographical location (Table 4), but the variables used in the edge model were those which could be considered to be associated with slow-flowing depositional areas (Table 4) and which are more likely to be affected by changes in water depth and flow rate. Thus, the set of environmental variables that were determined for use in the riffle model could be considered less changeable and marginally more robust than those determined for use in the edge model, suggesting that the riffle may be the most appropriate habitat for use in routine monitoring. However, it may be useful to develop predictive models for both edge and riffle habitats when the availability of riffle habitat in large lowland rivers is limited.

Habitat-specific sampling and assessment of biological impairment

The fauna in different habitats may respond variously to different water quality impacts. The riffle model allocated test sites 040 and 070 to band B (mildly impaired) but the edge model allocated them to band A (equivalent to reference; Table 6), probably because of the higher numbers of taxa generally collected from the latter habitat (Table 3). In both the riffle and edge models site 061 was allocated to band A (Table 6), indicating that the fauna collected from this test site was equivalent to reference conditions; this is in contrast to Norris (1994) who concluded that the site was impaired. However, site 061 is located immediately downstream of a native forested area, in the upper reaches of an otherwise urbanized catchment, and the fauna at this site may be less subject to disturbance than previously thought. When the outputs from models based on different habitats coincide more confidence can be placed in the conclusions. The potential application of predictive models to routine monitoring programmes in Australia is dependent upon the accurate assessment of the status of a test site, relative to reference conditions. Incorporation of the riffle and edge habitats in separate predictive models may sometimes result in the same site being allocated to different biological bands because of natural variation between the two habitats. In such cases, it may be appropriate to use the lower of the two bands because a more detailed investigation of biological condition at the site should be conducted before further action is taken.

Fewer taxa were collected from the pool-rock habitat at the test sites, than from the corresponding riffle or edge habitats (Table 3). However, it is unclear whether this is indicative of greater impairment of the pool fauna or whether it results from the different sampling method (100 animal live pick *v* 200 animal randomized subsample) used for the pool-rock habitat. Lenat (1988) found that the live pick method consistently gave a higher taxa richness compared with a kick net and

laboratory subsample procedure, and Storey *et al.* (1991) found that more taxa were collected in Surber samples than in kick net samples because sampling intensity was greater. In the habitats as individual objects model, pool-rock test site 061P was allocated to band C (Table 7) whereas the riffle and edge samples from the same site were allocated to band A in the same model (Table 7) and the riffle and edge models (Table 6). This suggests that, compared with the riffle and edge habitats, the pool-rock fauna at this site was impaired. However, Furse *et al.* (1981) could not establish whether differences in the abundance of taxa collected from different sites reflected actual variation in community structure or merely differing degrees of difficulty in catching each taxon under variable stream conditions. Thus, it may be that the substratum area covered by the pool-rock live pick was comparatively less than was covered by the dip net collection at other habitats, and this confounded the ability of the model to distinguish the variability between sites for the pool-rock habitat.

The major concern of limiting biological collections to a single habitat is that disturbances which manifest themselves in only certain habitats may go undetected (Kerans, Karr & Ahlstedt, 1992). In particular, it would be expected that the effects of sedimentation would be greater on the fauna of depositional areas than on the fauna of erosional areas (Hellawell, 1986; Hogg & Norris, 1991). The method used in this study to sample pool rocks limited collection to sites containing cobbles and boulders of a manageable size for live picking. Thus, the usefulness of pool rocks in testing for habitat-specific disturbances, such as sedimentation, was restricted. The majority of pool-rock reference sites were located in upland areas, biasing the faunal assemblages collected from this habitat and affecting the placement of lowland test sites into reference site groups based on upland faunal assemblages. Development of an accurate predictive model for the pool-rock habitat would require that all pools are sampled, regardless of substratum type. In the habitats as separate objects model, the limited number of pool-rock test sites were almost always placed into the same biological band as the corresponding riffle or edge sites in the same model (Table 7), the riffle or edge models (Tables 6 and 7) and the composite habitats model (Tables 7 and 8). This suggests that riffle and edge habitats are adequate to detect biological impairment and that the extra effort needed to sample pools may be unwarranted.

Many of the macrophyte beds (especially *Typha* and *Juncus* spp.) were located in the marginal areas, and often it was difficult to distinguish an edge habitat from a macrophyte habitat. There were no commonly occurring taxa which were unique to macrophytes (Table 3) and the two habitats can be accounted for by only sampling the edge, with no detrimental effect on the outcome of the predictive model. As with the pool rocks, the limited availability of discrete macrophyte habitats, their overlap with the edge, and the additional cost of sampling all justify elimination of this habitat from routine sampling in areas where riffle and edge habitats are commonly available.

Conclusion

All the models generally showed similar levels of impact at the test sites (Tables 6, 7 and 8), indicating a high level of redundancy among the habitat types sampled. Redundancy was also demonstrated by the lack of significant differences among observed/expected ratios generated by the various models. This suggests that only sufficient habitats need to be sampled to enable detection of biological impairment at test sites. In the region sampled in the present study this need was met by the riffle and edge habitats, both of which demonstrated biological impairment caused by a range of disturbance types (Table 1). Not only are additional habitats redundant, but also the inclusion of more than one habitat in a predictive model may cause confounded assessments of biological impairment. When habitats are included as individual objects, prediction of test sites into groups of equivalent reference sites is made according to the characteristics of a particular habitat type rather than general site features. When habitats are amalgamated into one sample the effect of varied sampling effort may also produce errors. It is also important that the habitat types used in predictive models should be represented throughout the study region. In the present study the riffle and edge habitats were the most commonly occurring habitats but in large lowland rivers, especially in Australia, edge and large woody debris might be more appropriate. The apparent robustness of the riffle habitat model and environmental prediction variables suggests that emphasis could be placed on this habitat, as has been advocated in other pro-

grammes (e.g. Plafkin *et al.*, 1989). Sampling habitats in addition to those needed to make accurate assessments of biological impairment is also a costly waste of resources.

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